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Patentanmeldung Nr. Patent application No. Demande de brevet n°

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Blatt 2 der Bescheinigung
Sheet 2 of the certificate
Page 2 de l'attestation

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Test animal for atherosclerosis model and methods for testing and screening

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Title: Test animal for atherosclerosis model and methods for testing and screening

Background of the invention

Atherosclerosis is a major problem in western society. Good model systems are essential to study the mechanism of atherosclerosis and the effects, if any, of potential anti-atherosclerotic drugs, diets or treatments. Most of the model systems used are based on animals in which atherosclerosis can be induced, in general by feeding them a cholesterol-rich diet over a prolonged period of time. Commonly used animal models are White New Zealand rabbits, Watanabe rabbits, and transgenic mice with disorders in the lipid metabolism (e.g. $ApoE^{-/-}$ mice, $LDL\text{-}R^{-/-}$ mice or ApoE3 Leiden mice).

The formation of atherosclerotic plaques in the vessel wall is a multi-step process that starts with the infiltration of monocytes into the vessel wall. These monocytes differentiate into macrophages in which lipid will accumulate. These lipid-loaded macrophages are called foam cells and result in the formation of a fatty streak in the vessel wall, the first stage of atherosclerosis.

This fatty streak will further develop into a mature atherosclerotic plaque by accumulation of fibrocellular mass consisting of smooth muscle cells, connective tissue and foam cells. Ultimately a mature atherosclerotic plaque will be formed that typically consists of a fibrous cap, lipid-loaded macrophages, smooth muscle cells, a broken internal elastic lamina and a necrotic core, possibly filled with cholesterol crystals.

Although in animal models the formation of atherosclerotic plaques can be induced, this process is rather slow. It takes usually several months to induce atherosclerosis, which is a major disadvantage if one wants to study the efficacy of potential anti-atherosclerotic drugs. For example in Watanabe rabbits, which are deficient in LDL-R, atherosclerosis develops only after 6 months (Aliev G, Burnstock G: Watanabe rabbits

with heritable hypercholesterolaemia: a model of atherosclerosis. Histol. Histopathol. 1998; 13: 797-817). White New Zealand rabbits develop atherosclerotic lesions after being fed a cholesterol-rich diet for at least 16 weeks. In transgenic mice having a disorder in the lipid metabolism (e.g. ApoE$^{-/-}$, LDL-R$^{-/-}$ or ApoE3 Leiden) it takes still three to six months to develop atherosclerotic lesions (Groot PHE, Van Vlijmen BJM, Benson GM, Hofker MH, Schiffelers R, Vidgeonhart M, Havekes LM: Quantitative assessment of aortic atherosclerosis in APOE*3 Leiden transgenic mice and its relationship to serum cholesterol exposure. Arteriosclerosis Thrombosis and Vascular Biology 1996; 16: 926-933; Hofker MH, van Vlijmen BJ, Havekes LM: Transgenic mouse models to study the role of ApoE in hyperlipidemia and atherosclerosis. Atherosclerosis 1998; 137: 1-11; Leppanen P, Luoma JS, Hofker MH, Havekes LM, Ylaherttuala S: Characterization of atherosclerotic lesions in apo E3-Leiden transgenic mice. Atherosclerosis 1998; 136: 147-152).

Although the expression of adhesion molecules on endothelium that overlies atherosclerotic plaques in ApoE$^{-/-}$ mice has been implicated in monocyte recruitment to developing lesions, monocyte adhesion in atherosclerotic vessels has not been observed (Ramos CL, Huo Y, Jung U, Ghosh S, Manka DR, Sarembock IJ, Ley K: Direct demonstration of P-selectin- and VCAM-1-dependent mononuclear cell rolling in early atherosclerotic lesions of apolipoprotein E-deficient mice. Circulation Research 1999; 84: 1237-1244).

Further, since atherosclerosis is a gradually proceeding process, it is difficult to determine the exact mechanisms of the early steps in atherosclerosis, the steps that should preferentially be blocked by interventional treatments.

Summary of the invention

The present invention deals with a new animal model in which atherosclerosis can be induced very rapidly in the vessel wall. By placement of a restrictive device around an artery in atherosclerosis prone animals the atherosclerotic plaque formation can be induced particularly at or near the place or spot of the restrictive device, the spot of cuff-placement.

Already within a couple of days foam cells can be detected and within two weeks full plaque formation can be seen, which is surprisingly fast. Such a novel procedure to induce atherosclerotic plaque formation in an extreme short time frame, at a predetermined site of the vessel wall, will substantially facilitate the in vivo efficacy analyses of possible anti-atherosclerotic drugs and treatments. Furthermore, since the atherosclerosis is induced by placement of a cuff, and thus the exact moment of induction and position of the formation of the atherosclerotic plaque is known, (effects of drugs on) the very early steps of atherosclerotic plaque formation can be studied.

It is observed that the placement of a collar around the carotid artery in rabbits has been reported to induce intimal hyperplasia, resulting in neointimal lesions, mainly consisting of smooth muscle cells. However, no accumulation of foam cells in the vessel wall surrounded by the collar could be observed (Kockx MM, Demeyer GR, Andries LJ, Bult H, Jacob WA, Herman AG: The endothelium during cuff-induced neointima formation in the rabbit carotid artery. Arterioscler. Thromb. 1993; 13: 1874-1884; Kockx MM, Demeyer GR, Jacob WA, Bult H, Herman AG: Triphasic sequence of neointimal formation in the cuffed carotid artery of the rabbit. Arterioscler. Thromb. 1992; 12: 1447-1457; Vanput DJM, Vanosselaer N, Demeyer GRY, Andries LJ, Kocks MM, Declerck LS, Bult H: Role of polymorphonuclear leukocytes in collar-induced intimal thickening in the rabbit carotid artery. Arterioscler. Thromb. Vasc. Biol. 1998; 18: 915-921; Soma MR, Donetti E, Parolini C, Sirtori CR, Fumagalli R, Franceschini G: Recombinant apolipoprotein A-IMilano dimer inhibits carotid intimal thickening induced by perivascular manipulation in rabbits. Circ. Res. 1995; 76: 405-411).

Similarly, placement of a cuff around the femoral artery of a wild-type mouse resulted in neointima formation consisting mainly of smooth muscle cells without accumulation of foam cells (Moroi M, Zhang L, Yasuda T, Virmani R, Gold HK, Fishman MC, Huang PL: Interaction of genetic deficiency of endothelial nitric oxide, gender, and pregnancy in vascular response to injury in mice. J. Clin. Invest. 1998; 101: 1225-1232).

Brief description of the drawings

Figure 1 illustrates neointima formation after 14 days, induced by cuff placement in the femoral artery of ApoE3 Leiden mice fed a normal chow diet (panel A) and a high-cholesterol diet (panel B). Note the lipid-loaden foam cells in the right panel.

Figure 2 gives a quantitative analysis of the neointima in cuffed femoral arteries of ApoE3 Leiden mice fed a cholesterol-rich diet N or standard chow diet. Neointima was measured in multiple sections of at least 3 mice and expressed as mean ± SEM.

Figure 3 illustrates the time course of atherosclerotic lesion formation in the femoral artery of ApoE3 Leiden mice after placement of a 0.4 mm polyethylene cuff. After one day first adhering monocytes can be observed, and foam cell accumulation can be observed already at day 3 after cuff placement. Foam cell accumulation gradually progresses the next days leading to a complete occlusion of the cuffed arteries already after 14 days.

Figure 4 illustrates foam cell accumulation in cuffed femoral arteries of mice with increasing serum cholesterol levels. In mice that received standard chow diet (cholesterol 2.13 mM), panel A, no foam cell accumulation could be observed, in diet W$^+$-fed mice (cholesterol 24.1 mM), panel B, moderate accumulation of foam cells could be observed and in diet N-fed mice (cholesterol 36.5 mM), panel C, massive accumulation of foam cells could be observed.

Detailed description of the invention

The subject invention provides a test animal useful in an animal model system for atherosclerosis, wherein the test animal is a non-human mammal susceptible to the induction of atherosclerosis which carries a vessel-restricting device applied to at least one of its blood vessels.

The words "vessel-restricting device" refer to a device which affects the freedom of movement of the blood vessels to which it is applied. The restriction of the vessel's freedom of movement may be quite minimal, such as a minimal restriction on

the freedom of the vessel to expand due to pulsation of the blood circulation. Although the shape of the device is not particularly limited, it is preferably ring-shaped, i.e. an annular device such as a cuff or ring. Preferably, the vessel-restricting device comprises a cuff or ring placed around part of a blood vessel of the animal. The inner diameter of the cuff or ring preferably corresponds roughly to the outer diameter of the blood vessel concerned. The device should not prevent blood flow through the vessel, but should contact the part of the vessel to which it is applied, at least during periods that the vessel expands due to pulsation of the blood flow.

The material of which the device is manufactured is not particularly relevant. Although e.g. metals and ceramics are suitable materials, plastics or polymeric materials are usually preferred. The material may be resorbable, but cheap materials such as polyethylene are fully satisfactory. So preferably, the vessel-restricting device is made of a plastic material, such as polyethylene. A simple ligament or suture would be useful as well.

The animal can be any mammal, with the exception of human beings. The phrase "susceptible to the induction of atherosclerosis" means that the animal has the potential to develop phenomena associated with atherosclerosis. An increased susceptibility to induction of atherosclerosis is not a requirement, but certainly preferred. Preferably, the animal is selected from the group consisting of monkey, pig, cow, sheep, goat, dog, horse, rabbit, hamster, Guinea pig, rat and mouse. More preferably, the animal is a rodent, in particular a mouse, most preferably a transgenic mouse with a disorder promoting its susceptibility to atherosclerosis, such as a disorder in its lipid metabolism promoting its susceptibility to atherosclerosis. In particular it is a transgenic mouse selected from the group consisting of $ApoE^{-/-}$, $LDL\text{-}R^{-/-}$ and ApoE3 mice.

The blood vessel is not particularly limited to certain blood vessels or types of blood vessels. Arteries and veins are useful, but preferably the vessel-restricting device is applied to an artery, such as femoral artery or carotic artery.

Normally, one vessel-restricting device will be applied to a part of one blood vessel. Another blood vessel or a different part of the same blood vessel may be used as a control. It is also possible, however, to provide more than one blood vessel, or more than one part of a blood vessel, with a vessel-restricting device, such as by placing cuffs around different blood vessels, or at different locations around the same blood vessel.

Furthermore, the subject invention provides a method for testing the atherosclerotic or anti-atherosclerotic effect of a substance, diet or treatment in an animal model system for atherosclerosis, comprising subjecting a test animal as defined herein to a test treatment with the substance, diet or treatment to be tested and analyzing the atherosclerotic or anti-atherosclerotic effect, if any, on the blood vessel restricted by the vessel-restricting device.

The test treatment to which the animal is to be subjected can have various forms. Normally, if the test seeks to examine the potential anti-atherosclerotic effect of a given substance, the test animal will receive a diet promoting the occurrence of atherosclerotic phenomena (e.g. a fatty or cholesterol-rich diet), together with the substance to be tested. Depending on the individual case, said substance may be administered orally, as part of the feed, or in the form of a separate medicament, or may be administered by other routes, such as parenterally, e.g. intravenously or intramuscularly. The route of administration is not particularly limited. To evaluate the effect of the substance to be tested, the test will normally include control test animals carrying a similar vessel-restricting device and fed with the same diet but without administration of the given substance.

If the test seeks to examine the potential anti-atherosclerotic effect of a given diet, the test animals will normally be fed with the diet to be tested and be compared with animals carrying a similar vessel-restricting device and fed with another diet, in particular a diet known to promote the occurrence of atherosclerosis. However, the test may also comprise feeding test animals first with a atherosclerosis-

promoting diet and subsequently with a diet to be tested, while control animals are kept on the atherosclerosis-promoting diet all the time, or are shifted to yet another feed selected as a comparative feed (to evaluate the anti-atherosclerotic effects of the diet to be tested in comparison to a given comparative diet).

Similar test formats may be used to evaluate the anti-atherosclerotic effect of a treatment to be tested.

The vessel-restricting device may be applied before the start of the test treatment, but it may also be applied after the start of the test treatment. The test animals may be fed with a atherosclerosis-promoting diet before application of the vessel-restricting device, or with a normal diet. The animals may receive the substance, diet or treatment to be tested, before application of the vessel-restricting device, or only after placement of the device. Preferably, the vessel-restricting device is applied to the at least one blood vessel after the start but before the end of the test treatment.

The analysis of the effect of the test treatment can be done in different ways, as well. The analysis may concentrate on the occurrence of monocytes adhering to endothelial cells, neointima formation or the accumulation of foam cells in (or in close proximity to) the restricted part of the blood vessel. Plaque formation is another phenomenon which may be selected for evaluation of the results. The test may look for partial or complete occlusion of the vascular lumen and even, although not preferred, for the development of a fibrous cap or necrotic core as usually occur in later stages of atherosclerosis.

So, as mentioned above, the method of the invention will preferably further comprise analyzing with the same animal, as a control, the atherosclerotic or anti-atherosclerotic effect, if any, on a blood vessel not restricted by the vessel-restricting device. Likewise preferably, the test treatment comprises administration to a test animal of a substance to be tested as anti-atherosclerotic agent together with an atherosclerosis-promoting diet, and wherein another test animal, fed with the same atherosclerosis-promoting diet but without the substance to be tested, is used as a control.

The subject invention is useful for screening purposes to identify substances, diets or treatments that are potentially beneficial for prophylaxis or treatment of atherosclerosis, so the subject invention also provides a method for screening substances, diets or treatments to identify one having an anti-atherosclerotic effect, comprising subjecting each of the substances, diets or treatments to be tested to a test in an animal model system for atherosclerosis in which a test animal as defined herein is subjected to a test treatment with the substance, diet or treatment to be tested and the anti-atherosclerotic effect, if any, on the blood vessel restricted by the vessel-restricting device is analyzed, and selecting a substance, diet or treatment having an anti-atherosclerotic effect.

The invention further provides a method for accelerating the onset and/or development of atherosclerotic phenomena in a non-human mammalian test animal comprising applying a vessel-restricting device to at least one of its blood vessels. In the absence of such vessel-restricting device, it may take months or longer before phenomena characteristic for atherosclerosis are observable, even when the test animal is fed with a diet known to induce atherosclerosis.

The invention also pertains to the use of a non-human mammalian test animal susceptible to the induction of atherosclerosis and carrying a vessel-restricting device applied to at least one of its blood vessels, for identifying substances, diets or treatments having an anti-atherosclerotic effect.

The present invention relates to the use of a restrictive device placed around a blood vessel to induce locally the accumulation of foam cells and as a consequence thereof eventually the formation of an atherosclerotic plaque. In particular this invention relates to placement of a cuff of a suitable diameter around a blood vessel in animals with an atherosclerotic phenotype. In particular this invention relates to placement of a cuff of a suitable diameter (0.1 - 2 mm) around a blood vessel in mice with an atherosclerotic phenotype. In particular this invention relates to placement of a polyethylene cuff around the femoral artery in transgenic mice.

In particular this invention relates to placement of a polyethylene cuff around the femoral artery in transgenic mice, these mice being mice in which a mutant form of the human ApoE3 gene, ApoE3 Leiden, is over-expressed (Van Vlijmen BJM, Van den Maagdenberg AMJM, Gijbels MJJ, Van der Boom H, Hogenesch H, Frants RR, Hofker MH, Havekes LM: Diet-Induced Hyperlipoproteinemia and Atherosclerosis in Apolipoprotein E3 Leiden Transgenic Mice. Journal of Clinical Investigation 1994; 93: 1403-1410). In these mice, when given a cholesterol-rich diet, without intervention atherosclerotic plaques can develop in 3 to 6 months.

The method described in the present invention can be used to test potential anti-atherosclerotic drugs and treatment-strategies. The term "anti-atherosclerotic" as used herein and in the claims will normally comprise a prevention of foam cell accumulation.

The invention addresses the solution of several negative aspects involved in use the animal models for atherosclerosis according to the prior art mentioned above:

- Fast induction of foam cell accumulation and atherosclerotic plaque formation can be obtained by placement of a poly-ethylene cuff around the femoral artery in atherosclerotic (transgenic) mice kept on a high-cholesterol diet for 2 to 4 weeks. Foam cell accumulation starts already within the first three days after cuff placement and within 14 days the atherosclerosis in the treated vessel can progress to such an extent that complete occlusion of the vascular lumen can be obtained. The atherosclerotic transgenic mice can be for example ApoE3 Leiden mice, LDL-$R^{-/-}$ mice, or $ApoE^{-/-}$ mice, but without restriction thereto. Furthermore, cuff placement is not restricted to the femoral artery, but other vessels e.g. the carotic artery or the abdominal aorta are equally suitable.
- The foam cell accumulation and atherosclerotic plaque formation predominantly occurs within the vessel segment, which has the advantage that the atherosclerosis occurs at a location that is exactly known. Furthermore, contralateral untreated blood vessels of the same animal can be used as

controls in the studies.

- The foam cell accumulation in the vessel segment surrounded by the cuff starts directly after cuff placement. Monocyte adhesion to the endothelial cells in the treated blood vessel can be observed already one day after cuff placement. This makes the model system described in this invention extremely suitable to study the mechanism of early plaque formation and the effect of potential drugs on this process. In the model systems described in the prior art this is extremely difficult, if not impossible, since in these models the atherosclerosis process progresses more slowly and the exact moment of induction and location of the atherosclerosis is not known.
- The present invention is highly suitable to study the early steps in atherosclerotic plaque formation. For studying the later stages in plaque formation, the development of a fibrous cap, necrotic core formation etc., the presented model system is less suitable. However, accelerated atherosclerosis observed in vein grafts (less organized structures lacking typical features such as the fibrous cap and necrotic core) shows high morphological resemblance with the atherosclerosis observed in this model.

The present application will be described herein in further detail, while referring to the following examples. It is to be noted that these examples merely serve to illustrate the invention, not to restrict it.

Example 1

Transgenic mice carrying the ApoE3Leiden gene (male, 8-12 weeks of age) were kept on a cholesterol-rich diet for 4 weeks (Diet N: 20% casein, 1% choline chloride, 0.2% methionine, cocoa butter 15%, cholate 0.5%, cholesterol 1%, sucrose 40.5%, cornstarch 10%, corn oil 1%, cellulose 5.1%, mineral mixture 5.1%; all percentages are in weight/weight). After these 4 weeks a polyethylene cuff (0.4 mm diameter) was placed around the femoral artery of these mice according to a procedure described by Moroi et al. (Moroi M, Zhang L, Yasuda T, Virmani

R, Gold HK, Fishman MC, Huang PL: Interaction of genetic deficiency of endothelial nitric oxide, gender, and pregnancy in vascular response to injury in mice. J. Clin. Invest. 1998; 101: 1225-1232). The diet was continued. Two weeks after cuff placement mice were killed and histological analysis was performed. In the mice receiving the atherosclerosis-inducing cholesterol-rich diet N, abundant accumulation of foam cells in the vessel wall of the cuff surrounded segment can be observed (Fig. 1). In the contralateral control vessel no neointima formation or foam cell accumulation could be observed. In the vessel wall of the control mice that received a standard chow diet (both before and after placing the cuff), in the vessel wall a neointimal layer consisting of smooth muscle cells but no foam cells could be observed.

Quantitative analysis of the total neointima formed in these mice demonstrated a 3.75 ± 0.8 fold increase in neointima in the mice that were fed a cholesterol-rich diet (diet N) when compared to the mice receiving standard chow (mean neointima 8096 μm^2 for diet N vs 2158 μm^2 for standard chow), see Fig. 2.

Example 2

Transgenic mice carrying the ApoE3 Leiden gene (male, 8-12 weeks of age) were kept on a cholesterol-rich diet for 4 weeks (Diet N: 20% casein, 1% choline chloride, 0.2% methionine, cocoa butter 15%, cholate 0.5%, cholesterol 1%, sucrose 40.5%, cornstarch 10%, corn oil 1%, cellulose 5.1%, mineral mixture 5.1%; all percentages are in weight/weight). After these 4 weeks a polyethylene cuff (0.4 mm diameter) was placed around the femoral artery of these mice according the procedure described above. The diet was continued. Mice were killed one day, three days, 7 days, 10 days and 14 days after cuff placement and histological analysis was performed. In the mice receiving the atherosclerosis-inducing cholesterol-rich diet N, already one day after cuff placement adhering monocytes could be observed at the endothelial cells, whereas in the normal diet counterparts no such effects could be observed. After three days foam cells could be detected in the vessel wall of the cuff-surrounded segment. This foam cells accumulation

continued in the next days and resulted in a nearly complete occlusion of the treated vessel after 10 to 14 days (Fig. 3).

Example 3

Transgenic mice carrying the ApoE3 Leiden gene (male, 8-12 weeks of age) were kept on a cholesterol-rich diet for 4 weeks (Diet N: 20% casein, 1% choline chloride, 0.2% methionine, cocoa butter 15%, cholate 0.5%, cholesterol 1%, sucrose 40.5%, cornstarch 10%, corn oil 1%, cellulose 5.1%, mineral mixture 5.1%; all percentages are in weight/weight) or a less severely cholesterol-raising diet (Diet W$^+$: 20% casein, 1% choline chloride, 0.2% methionine, cocoa butter 15%, cholate 0.1%, cholesterol 1%, sucrose 40.5%, cornstarch 10%, corn oil 1%, cellulose 4.7%, mineral mixture 5.1%; all percentages are in weight/weight). After these 4 weeks a polyethylene cuff (0.4 mm diameter) was placed around the femoral artery of these mice as described above. The diets were continued. Two weeks after cuff placement mice were killed, serum-cholesterol levels were determined and histological analysis of the neointima and atherosclerotic plaque formation was performed. In these groups of mice the extent of atherosclerotic plaque formation c.q. foam cell accumulation correlates with the serum-cholesterol levels. Fig. 4 shows the foam cell accumulation in cuffed femoral arteries of mice with increasing serum-cholesterol levels. In mice that received standard chow diet (cholesterol 2.13 mM), panel A, no foam cell accumulation could be observed, in diet W$^+$-fed mice (cholesterol 24.1 mM), panel B, moderate accumulation of foam cells could be observed and in diet N-fed mice (cholesterol 36.5 mM), panel C, massive accumulation of foam cells could be observed.

Example 4

Transgenic mice carrying the ApoE3 Leiden gene (male, 8-12 weeks of age) were kept on a less severely cholesterol-raising diet (Diet W: 20% casein, 1% choline chloride, 0.2% methionine, cocoa butter 15%, cholesterol 1%, sucrose 40.5%, cornstarch 10%, corn oil 1%, cellulose 4.7%, mineral mixture 5.1%; all percentages are in weight/weight) with or without 0.015%

atorvastatin. After these 4 weeks a polyethylene cuff (0.4 mm diameter) was placed around the femoral artery of these mice as described above. Two weeks after cuff placement mice were killed, serum-cholesterol levels were determined and histological analysis of the neointima and atherosclerotic plaque formation was performed. In the mice receiving the atorvastatin treatment cholesterol levels were reduced by 60%. Furthermore, in these groups of mice the extent of atherosclerotic plaque formation c.q. foam cell accumulation correlates with the serum-cholesterol levels.

Example 5

In transgenic ApoE$^{-/-}$ mice a polyethylene cuff (0.4 mm diameter) was placed around the femoral artery as described above. Directly after cuff placement these mice were infected with an adenoviral vector expressing human ApoE. This treatment resulted as previously reported (Van Dijk KW, van Vlijmen BJ, van't Hof HB, van der Zee A, Santamarina-Fojo S, van Berkel TJ, Havekes LM, Hofker MH: In LDL receptor-deficient mice, catabolism of remnant lipoproteins requires a high level of apoE but is inhibited by excess apoE. J. Lipid Res. 1999; 40: 336-344) in a reduction of serum-cholesterol levels from 35.2 ± 6.7 to 14.6 ± 2.3 mmol/l. Two weeks after cuff placement mice were killed, serum-cholesterol levels were determined and histological analysis of the neointima and atherosclerotic plaque formation was performed. A cholesterol-dependent reduction of neointima formation c.q. foam cell accumulation could be observed.

CLAIMS

1. A test animal useful in an animal model system for atherosclerosis, wherein the test animal is a non-human mammal susceptible to the induction of atherosclerosis which carries a vessel-restricting device applied to at least one of its blood vessels.

2. A test animal according to claim 1, wherein said vessel-restricting device comprises a cuff or ring placed around part of a blood vessel of the animal.

3. A test animal according to claim 1 or 2, wherein said vessel-restricting device is made of a plastic material, such as polyethylene.

4. A test animal according to any one of claims 1-3, wherein said animal is selected from the group consisting of monkey, pig, cow, sheep, goat, dog, horse, rabbit, hamster, Guinea pig, rat and mouse, preferably is a rodent, most preferably mouse.

5. A test animal according to claim 4, wherein said animal is a transgenic mouse with a disorder promoting its susceptibility to atherosclerosis, more particularly a disorder in its lipid metabolism promoting its susceptibility to atherosclerosis.

6. A test animal according to claim 5, wherein said transgenic mouse is selected from the group consisting of $ApoE^{-/-}$, $LDL\text{-}R^{-/-}$ and ApoE3 mice.

7. A method for testing the atherosclerotic or anti-atherosclerotic effect of a substance, diet or treatment in an animal model system for atherosclerosis, comprising subjecting a test animal as defined in any one of claims 1-6 to a test treatment with the substance, diet or treatment to be tested and analyzing the atherosclerotic or anti-atherosclerotic

effect, if any, on the blood vessel restricted by the vessel-restricting device.

8. A method according to claim 7, wherein the vessel-restricting device is applied to the at least one blood vessel after the start but before the end of the test treatment.

9. A method according to claim 7 or 8, further comprising analyzing with the same animal, as a control, the atherosclerotic or anti-atherosclerotic effect, if any, on a blood vessel not restricted by the vessel-restricting device.

10. A method according to any one of claims 7-9, wherein said test treatment comprises administration to a test animal of a substance to be tested as anti-atherosclerotic agent together with an atherosclerosis-promoting diet, and wherein another test animal, fed with the same atherosclerosis-promoting diet but without the substance to be tested, is used as a control.

11. A method for screening substances, diets or treatments to identify one having an anti-atherosclerotic effect, comprising subjecting each of the substances, diets or treatments to be tested to a test in an animal model system for atherosclerosis in which a test animal as defined in any one of claims 1-6 is subjected to a test treatment with the substance, diet or treatment to be tested and the anti-atherosclerotic effect, if any, on the blood vessel restricted by the vessel-restricting device is analyzed, and selecting a substance, diet or treatment having an anti-atherosclerotic effect.

12. A method for accelerating the onset and/or development of atherosclerotic phenomena in a non-human mammalian test animal comprising applying a vessel-restricting device to at least one of its blood vessels.

13. Use of a non-human mammalian test animal susceptible to the induction of atherosclerosis and carrying a vessel-restricting device applied to at least one of its blood

vessels, for identifying substances, diets or treatments having an anti-atherosclerotic effect.

ABSTRACT

Title: Test animal for atherosclerosis model and methods for testing and screening

A test animal useful in an animal model system for atherosclerosis, wherein the test animal is a non-human mammal susceptible to the induction of atherosclerosis which carries a vessel-restricting device applied to at least one of its blood vessels. A method for testing the (anti-)atherosclerotic effect of a substance, diet or treatment in an animal model system for atherosclerosis, comprising subjecting a test animal according to the invention to a test treatment with the substance, diet or treatment to be tested and analyzing the (anti-)atherosclerotic effect, if any, on the blood vessel restricted by the vessel-restricting device. The method may be used for screening potentially useful substances, diets or treatments.

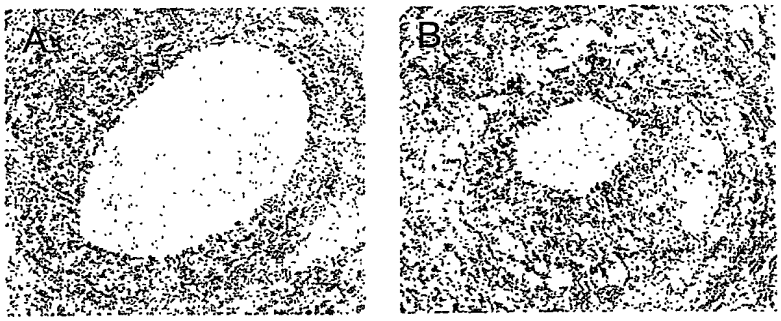
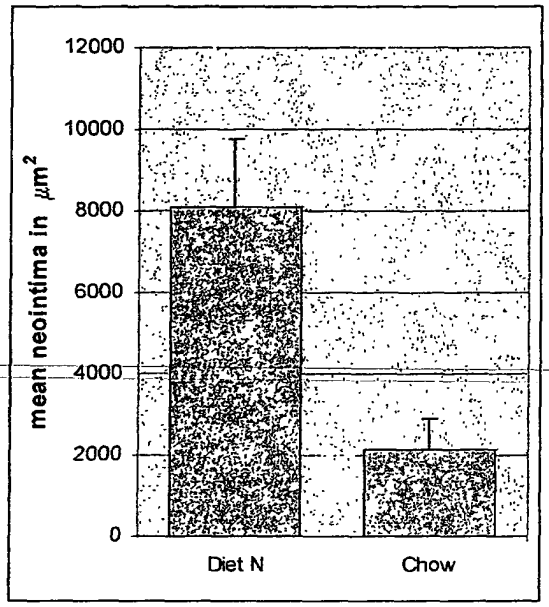


Figure 1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8000
6000
4000
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mean neointima in μm^2
Diet N
Chow

Figure 2

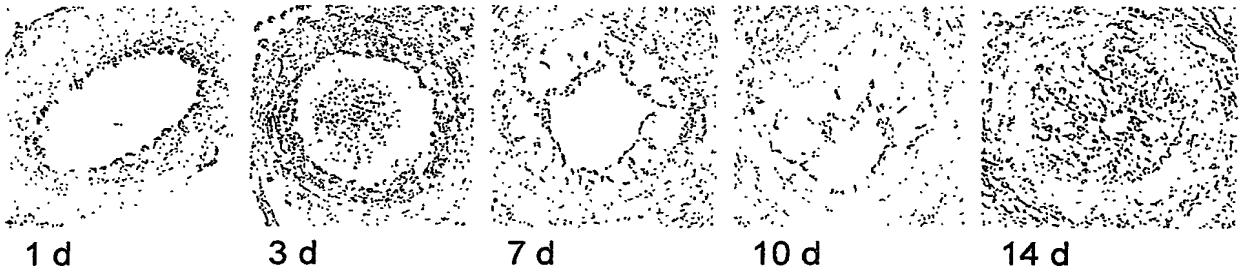

Figure 3

Figure 4